ABSTRACT

Provided is a method of producing a template DNA used for protein synthesis comprising a step of amplifying a linear double-stranded DNA by polymerase chain reaction (PCR), by using a reaction solution comprising a first double-stranded DNA fragment comprising a sequence coding for a protein or a portion thereof, a second double-stranded DNA fragment comprising a sequence overlapping with the 5' terminal region of the first DNA fragment, a third double-stranded DNA fragment comprising a sequence overlapping with the 3' terminal region of the first DNA fragment, a sense primer which anneals with the 5' terminal region of the second DNA fragment, and an anti-sense primer which anneals with the 3' terminal region of the third DNA fragment, wherein the second DNA fragment comprises a regulatory sequence for transcription and translation of a gene, and the concentrations of the second DNA fragment and the third DNA fragment in the reaction solution each range from 5 to 2,500 pmol/L. The use of this method enables efficient production of a template DNA for expression and purification of a protein.